

## Effect of growth intensity of bulls on the microstructure of musculus longissimus lumborum and meat quality

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### Abstract

The aim of this study was to evaluate the effect of growth intensity of 43 bulls with different growth intensity (< 900 and ≥ 900 g/day) on the microstructure of musculus longissimus lumborum. Commercial crosses of Polish Lowland black-and-white cows with Charolais and Limousin bulls were used in this study; within the particular genetic groups the hybrids had similar slaughter weight (447.6 and 517.2 kg) and age (526 and 606 days), respectively. The share of fibres with active tetrazole dehydrogenase in the more intensively growing animals was smaller. For fibres with myofibrillar ATPase activity, the intensively growing animals produced higher standard deviation values than the other groups. Further analysis of the muscular tissue in this group revealed that out of the 24 muscles, 9 had giant fibres. In comparison with the less intensively growing animals, the muscles of the bulls that gained more than 900 g/day in weight were found to contain significantly less glycogen ( $P \leq 0.01$ ) and, consequently, the meat was less acidic. The difference of the pH ranged from 0.19 in the case of pH<sub>24</sub> ( $P \leq 0.01$ ) to 0.06 for pH<sub>48</sub> ( $P \leq 0.01$ ). It should be noted that the intensively growing animals were found to have a relatively high pH variability (SD = 0.69 and 0.49, respectively). The pH<sub>24</sub> and pH<sub>48</sub> values, as well as pH variability show that the meat of this group was dark, firm and dry.

*Daily body gain, beef, histological traits, meat faults*

One of the most effective methods to increase beef production is using commercial cross-breeding for which Charolais and Limousin bulls are predominantly used at present. On the other hand, satisfactory results could be obtained with production efficiency and the possibility of using growth-intensifying feeds for fattening the hybrids. Growth intensity and commercial cross-breeding significantly affect the formation muscle microstructure and muscle metabolism. As a result, the processing and cooking usefulness of meat can be enhanced (Ozawa et al. 2000; Vestergaard et al. 2000; Wegner et al. 2000; Młynek and Guliński 2007).

Meat quality is predominantly affected by the feeding regime, growth potential, body weight and age of slaughtered cattle (Młynek et al. 2006; Enderr 2008). Such characteristics of meat as tenderness, water retention capacity, colour or acidity are related to the structure of intravitaly developed muscles (Młynek et al. 2006; Keady et al. 2007) and have an enormous importance for the consumer and the processing industry.

The aim of this study was to identify the influence of growth intensity of bulls on musculus longissimus lumborum microstructure characteristics and verify whether growth intensity affects the metabolic profile, meat acidification and incidence of quality defects.

### Materials and Methods

The study was performed on 43 hybrids (the mean age was 566 days), crosses of Polish Lowland black-and-white breed cows (BW) in which the gene share of the Holstein Friesian breed did not exceed 25% with bulls of Charolais breed (CHAR) (n = 22) or Limousine breed (LIM) (n = 21). Bulls were kept under similar conditions but came from different farms. Fattening started when the calves weighed 150–180 kg.

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In terms of gain and fattening time, growth intensity was calculated ( $GI_{g/day}$ ). Bulls were divided into two groups: bulls with grow intensity of  $< 900$  g/day and bulls with grow intensity  $\geq 900$  g/day. In the autumn–winter period, animals from the first group were fed hay *ad libitum* and corn silage (approximately 10 kg/24 h). In the summer, green fodder and straw were provided *ad libitum*. Compound cereal meal was used as a supplement to the main diet for animals in the second group at approximately 1.0 kg/24 h throughout the fattening.

After a 24-h rest, the bulls were weighed and then slaughtered according to slaughterhouse procedures. Samples for histochemical analysis were collected within 30 min after slaughter, from the middle part of musculus longissimus lumborum. Samples were immediately cut into  $1 \times 1$  cm pieces (parallel to the muscle fibres) and frozen using liquid nitrogen.

The cryopreparations were made using the SHANDON OT cryostat at  $-25$  °C and cut into 10  $\mu$ m-thick slices. The muscle microstructure assessment was conducted according to the method analysing the enzymatic activity of fibres (Dubovitz et al. 1973). Histochemical analysis differentiated muscle fibres into Slow-twitch Oxidative (STO), Fast-twitch Oxidative-Glycolytic (FTO) and Fast-twitch Glycolytic (FTG). Fibres STO (night-blue) and FTO (blue) were identified based on tetrazole dehydrogenase activity (NADH-TR) after preincubation in a pH 4.0 buffer with NADH. Grey-dyed FTG fibres were identified based on myofibrillar ATP-ase activity after incubation in a pH 9.6 buffer.

Analysis of the muscle microstructure profile was performed based on the measurements recorded for 10 randomly selected fascicles of each muscle. The analysis included measurements of muscle fibre cross section area ( $\mu$ m<sup>2</sup>) and calculations of mean fragmentation area ( $\mu$ m<sup>2</sup>) and mean fibre type distribution (%). Using the area and frequency of fibre types, the relative area of fibre type was determined and then the aerobic –  $AF\% = [(STO+FTO)/FTG]$  and anaerobic indices –  $AnF\% = [(FTG+FTO)/STO]$  were calculated.

The presence of glycogen (%) in tissue was determined based on the histochemical periodic acid-schiff colour reaction. Taking advantage of colour intensity increase with rising glycogen (Šrutek and Kłosowska 2005). The assay technique consisted of analysing the microscopic image and measuring colour intensity.

The acidity in tissue was proceeded 24 h (pH<sub>24</sub>) and 48 h (pH<sub>48</sub>) *post mortem*.

The muscle structure and glycogen content were identified on the basis of images obtained with OLYMPUS BX41 compatible with the OLYMPUS Soft Imaging System – Cell<sup>®</sup>.

Statistical data were processed with STATISTICA 9.0, providing the following: mean values ( $\bar{X}$ ), standard deviations (SD) and values for variance analysis in a non-orthogonal design. The population profile included evaluation of differences between the following mean values: slaughter weight, age and daily weight gains in the genetic groups under analysis. A model allowing for growth intensity was used for the analysis of the microstructure and the metabolic indices. The significance of differences was assessed using the Duncan multiple range test ( $P \leq 0.01$ ,  $P \leq 0.05$ ).

## Results

Data presented in Table 1 show that in the case of the slaughter weight, age and growth intensity, differences between commercial hybrids were small and non-significant. On the other hand, significant differences ( $P \leq 0.05$ ) were confirmed for the values of slaughter indicators between animal categories with different growth intensities.

Animals with weight gains of 992 and 973 g/day (the  $\geq 900$  g/day category) had 70 kg greater body weights with the average body weight of 517.2 kg and were younger by about 79 days (the mean age was 526.6 days).

Table 1. Characteristics of slaughter indices and daily body gain of bulls ( $\bar{X} \pm$  SD)

Growth intensity categories (g/day)	Slaughter indices					
	Slaughter weight (kg)		Age (day)		Growth intensity (g/day)	
	BW × CHAR	BW × LIM	BW × CHAR	BW × LIM	BW × CHAR	BW × LIM
(n)	(22)	(21)	(22)	(21)	(22)	(21)
$\leq 900$ (n = 19)	445.0 <sup>a</sup> ± 37	450.2 <sup>a</sup> ± 55	597 <sup>a</sup> ± 32	615 <sup>a</sup> ± 24	745 <sup>a</sup> ± 42	732 <sup>a</sup> ± 51
$\geq 900$ (n = 24)	516.8 <sup>b</sup> ± 44	517.6 <sup>b</sup> ± 38	521 <sup>b</sup> ± 21	532 <sup>b</sup> ± 41	992 <sup>b</sup> ± 35	973 <sup>b</sup> ± 44
Total (n = 43)	481.0 ± 41	483.9 ± 45	559 ± 27	573 ± 32	873 ± 38	852 ± 47

BW - Polish Lowland black-and-white breed, CHAR - Charolais, LIM - Limousine breed

<sup>abc</sup>- significant differences in columns ( $P \leq 0.05$ )

Table 2. Characteristics ( $\bar{x} \pm SD$ ) of microstructure of *m. longissimus lumborum* in bulls in two different growth intensity categories

Estimation traits	Growth intensity categories (g/day)		
	$\leq 900$ (n = 19)	$\geq 900$ (n = 24)	Total (n = 43)
Fibre number	35.3 $\pm$ 4.3	38.6 $\pm$ 6.2	36.9 $\pm$ 5.3
Mean fragmentation area ( $\mu\text{m}^2$ )	1685 <sup>A</sup> $\pm$ 134	1818 <sup>B</sup> $\pm$ 211	1752 $\pm$ 173
Cross section area ( $\mu\text{m}^2$ ):	STO	1655 $\pm$ 132	1673 $\pm$ 137
	FTO	1661 <sup>a</sup> $\pm$ 149	1701 $\pm$ 174
	FTG	1741 <sup>A</sup> $\pm$ 121	1922 <sup>B</sup> $\pm$ 293
Fibre types distribution (%):	STO	22.2 <sup>A</sup> $\pm$ 1.4	21.2 $\pm$ 1.9
	FTO	19.5 <sup>a</sup> $\pm$ 1.8	20.1 $\pm$ 2.6
	FTG	58.3 <sup>a</sup> $\pm$ 1.7	59.1 <sup>b</sup> $\pm$ 4.5
Number of giant fibre (n)	NF	1.2* $\pm$ 0.6	-
Frequency of giant fibre (%)	NF	2.9* $\pm$ 0.4	-

NF – not found; \*9 animals, STO - Slow-twitch oxidative, FTO - Fast-twitch oxidative-glycolytic, FTG - Fast-twitch glycolytic.

Significant differences in rows: <sup>abc</sup>  $P \leq 0.05$ , <sup>ABC</sup>  $P \leq 0.01$ .

Table 3. Characteristics ( $\bar{x} \pm SD$ ) of metabolism, amount of glycogen and pH in *m. longissimus lumborum* of two growth intensity categories of bulls

Traits	Growth intensity categories (g/day)		
	$< 900$ (n = 19)	$\geq 900$ (n = 24)	Total (n = 43)
AF (%)	0.743 <sup>a</sup> $\pm$ 0.135	0.622 <sup>b</sup> $\pm$ 0.122	0.683 $\pm$ 0.128
AnF (%)	3.613 <sup>a</sup> $\pm$ 0.157	3.874 <sup>b</sup> $\pm$ 0.173	3.744 $\pm$ 0.165
Glycogen (%)	56.86 <sup>A</sup> $\pm$ 4.51	51.14 <sup>B</sup> $\pm$ 3.71	54.00 $\pm$ 4.11
pH <sub>24</sub>	5.78 <sup>A</sup> $\pm$ 0.15	5.97 <sup>B</sup> $\pm$ 0.69	5.88 $\pm$ 0.42
pH <sub>48</sub>	5.57 <sup>a</sup> $\pm$ 0.10	5.63 <sup>b</sup> $\pm$ 0.49	5.60 $\pm$ 0.30

AF – aerobic indices, AnF – anaerobic indices

Significant differences in rows: <sup>abc</sup>  $P \leq 0.05$ , <sup>ABC</sup>  $P \leq 0.01$ .

Table 2 shows the results for changes in the microstructure of *m. longissimus lumborum* of bulls in the two growth intensity categories. It can be seen that the mean fibre area of the more intensively growing animals was larger by 133  $\mu\text{m}^2$  ( $P \leq 0.01$ ). The difference in the size of the determined fibre types predominantly concerned fibres with myofibrillar ATPase activity (FTG); the difference amounted to 181  $\mu\text{m}^2$  ( $P \leq 0.01$ ).

A slightly smaller difference of 80  $\mu\text{m}^2$  ( $P \leq 0.05$ ) was identified for FTO fibres. Growth intensity also influenced the developing proportions between the fibre types. Muscles of the intensively growing bulls had a smaller (by about 2.2%) share of fibres with active dehydrogenase. Differences in the share of the anaerobic (both FTO and FTG) fibres were not so prominent and ranged from 0.8 to 1.3%.

The greatest variability in the cross-section area (SD of 199 and 293) and percentage share (SD of 3.5 and 4.5) was identified for anaerobic (FTG) and intermediary (FTO) fibres. This suggests that the muscles of the animals in the  $\geq 900$ g/day category contained fibres with greater cross-section areas than the population average. This theory was eventually confirmed by the data in Table 2 that show the presence of giant fibres in the more intensively growing animals. Such fibres were found to be present in 9 muscles (37.5%) of all the muscles in this category of bulls.

Further effect of growth intensity on the bull muscle metabolism, acidity and major meat quality criteria are shown in Table 3.

The aerobic and anaerobic indices show predominance of anaerobic metabolic transformations consisting of muscle glycogen break-up in the muscles of the intensively growing animals. In comparison with the muscles of the bulls whose daily weight gain (group  $\leq 900$ ) ranged from 745 to 732 g/day, 30 min after slaughter the glycogen content

in the muscular tissue of the intensively growing animals (group  $\geq 900$ ) was lower as evidenced in less intense redness (%) of the preparations. The relevant value was lower by 5.71 units. The microstructure changes and lower glycogen supply resulted in lower acidification of the muscles in this group:  $\text{pH}_{24} = 5.97$  ( $P \leq 0.01$ ) and  $\text{pH}_{48} = 5.63$  ( $P \leq 0.05$ ).

The results of standard deviation obtained for the pH of the meat of the intensively growing bulls with giant fibres (SD = 0.69 for  $\text{pH}_{24}$  and 0.49 for  $\text{pH}_{48}$ ) suggest typical acidity of dark firm and dry meat. Therefore, this part of research requires further analyses to specify whether the lower meat acidity was exclusively associated with excessive hypertrophy.

### Discussion

The diet and postnatal changes in the muscular tissue significantly affect beef quality development and the efficiency of increasing the carcass meat content. According to Ashmore et al. (1974) and Wegner et al. (2000), an essential role in this respect is played by the share of fibres with different metabolism. The influence of fibre structure (the share of FTG fibres) on meat acidity was also observed by Rehfeldt et al. (2000). Kim et al. (2000) reported that meat acidification does not entirely depend on the glycogen content but also on the share of glycolytic fibres. They found that the acidification of *m. psoas major* ( $\text{pH}_{24} = 5.61$ ) was faster than that of *m. longissimus dorsi* ( $\text{pH}_{24} = 5.73$ ) which contained less glycogen but had a greater share of glycolytic fibres. Vestergaard et al. (2000) and Młynek et al. (2006) have reported a similar pattern.

Hoch et al. (2005) revealed a higher share of fast glycolytic (FG) fibres in the *m. triceps brachii* (55.8–57.4%) of intensively growing animals (from 884 to 975 g/day). The percentage share of these fibres in the less intensively growing animals (558–698 g/day) ranged from 54.5 to 55.5%, while their activity of lactate dehydrogenase and isocitrate dehydrogenase was significantly lower. A similar pattern in the development of the percentage share of fibres with different enzymatic activity was suggested by Ouali (1990), Wegner et al. (2000) or Młynek and Guliński (2007).

Ozawa et al. (2000) did not confirm a direct effect of growth intensity on the post-slaughter meat acidity profile. However, they identified significant correlation between growth intensity and fibre structure and size. The greatest aW fibre diameter (55.6  $\mu\text{m}$ ) and share (53.4%) was identified in animals whose weight gain amounted to 0.69 kg/day. The above researchers observed a negative correlation ( $r = -0.31$ ) between the magnitude of daily weight gains and meat acidification in the case of these fibres.

A major quality discriminant of meat is its pH which largely depends on post-slaughter glycogenolytic processes. According to Immonen et al. (2000), 45 mmol/kg of glycogen reduces the pH of meat from 7.2 to 5.5. Glycogen accumulation efficiency depends on individual characteristics. However, it is also affected by the feeding quality and pre-slaughter conditions (stress).

As shown in a study by Vestergaard et al. (2000), more intensively fed bulls slaughtered at lower body weight have, as a rule, higher glycogen content. The authors also proved that an extensive feeding regime is conducive to an increase of aerobic metabolism and higher activity of lactic acid dehydrogenase.

On the other hand, results obtained by Wegner et al. (2000) and Młynek and Guliński (2007) indicate a greater share and increased oxidative activity of muscle fibres in older cattle. Ashmore (1974) and Young and Foote (1984) suggested that a high oxidative metabolic activity of fibres can lead to lower acidity and a darker meat colour, both associated with the DFD defect.

In the case of cattle, faster glycolysis is more common in more muscular and heavier animals. Additionally, a greater share of glycolytic fibres facilitates pre-slaughter glycogen break-up and inhibits lactic acid accumulation after slaughter. This may suggest that more

intensively growing animals are prone to faster glycogen depletion and lower meat acidity after slaughter.

In conclusion, enhancing growth intensity of commercial hybrids derived from Charolais and Limousin bulls is associated with changes in the cross section area and the share of fibres with higher myofibrillar ATPase activity. The changes result in predominance of anaerobic metabolism and smaller glycogen accumulation in the muscles of intensively growing animals.

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